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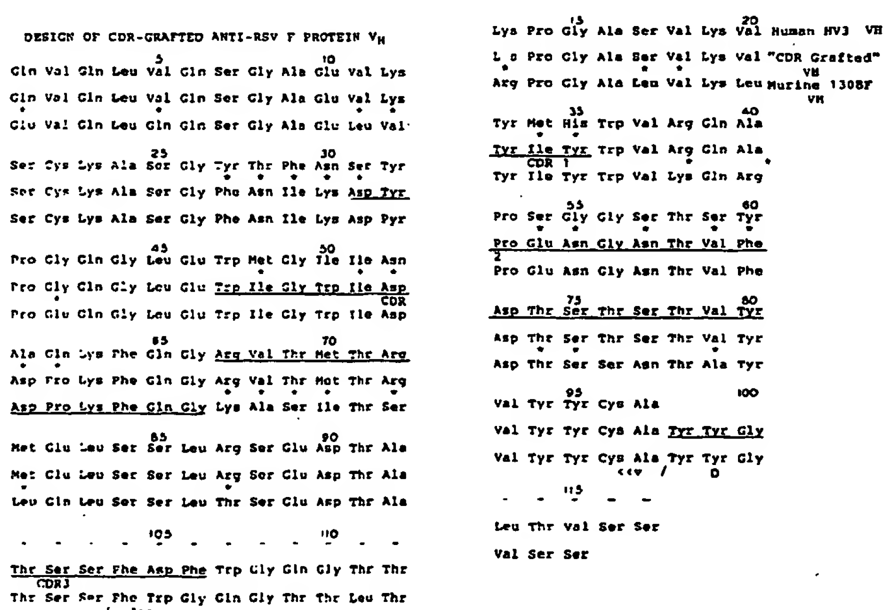

(57) Abstract

This invention relates to a human antibody which contains at least one CDR from each variable heavy and variable light chain of at least one murine monoclonal antibody, (preferably a neutralizing antibody) against respiratory syncytial virus. The invention also relates to the murine antibody being an antibody against RSV-F protein. In a preferred embodiment the CDR contains three complementarity determining regions from the variable heavy and variable light chain of at least one murine antibody against RSV-F protein.

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Human-Murine Chimeric Antibodies Against Respiratory Syncytial Virus

BACKGROUND

Respiratory syncytial virus (RSV) is the major cause of acute respiratory illness in young children admitted to hospitals, and the community practice will treat perhaps five times the number of hospitalized children. It is therefore, the most common cause of lower respiratory tract infection in young children. While the majority of community-acquired infections resolve themselves in a week to ten days, many hospitalized children, especially under six months of age require assisted ventilation.

Efforts to produce an effective vaccine have been unsuccessful (8). A major obstacle to vaccine development is safety; the initial formalin inactivated RSV vaccine caused an increased incidence of RSV lower respiratory tract disease and death in immunized children upon exposure to virus (5).

Recently, the drug ribavirin has been licensed for therapy of RSV pneumonia and bronchiolitis (2,3); its value is contraversial (4). Although ribavirin has shown efficacy (9), the drug has to be administered over an 18 hour period by aerosol inhalation. In addition, the level of secondary infections following cessation of treatment is significantly higher than in untreated patients.

Studies have shown that high-titered RSV immunoglobulin was effective both in prophylaxis and therapy for RSV infections in animal models (6, 7). Infected animals treated with RSV immune globulin, showed no evidence of pulmonary immune-complex disease (6, 7).

Even if RSV hyperimmune globulin is shown to reduce the incidence and severity of RSV lower respiratory tract infection in high risk children, several disadvantages may

limit its use. One drawback is the necessity for intravenous infusion in these children who have limited venous access because of prior intensive therapy. A second disadvantage is the large volume of RSVIG required for protection, particularly since most these children have compromised cardiopulmonary function. A third disadvantage is that intravenous infusion necessitates monthly hospital visits during the RSV season which places these children at risk of nosocomial RSV infection (1). A final problem is that it may prove to be very difficult to select sufficient donors to produce a hyperimmune globulin for RSV to meet the demand for this product. Currently only about 8% of normal donors have RSV neutralizing antibody titers high enough to qualify for the production of hyperimmune globulin.

Another approach may be the development of monoclonal antibodies with high specific neutralizing activity as an alternative to hyperimmune globulin. It is preferable, if not necessary, to use human monoclonal antibodies rather than murine or rat antibodies to minimize the development of human anti-rodent antibody responses which may compromise the therapeutic efficacy of the antibody or induce immune-complex pathology. However, the generation of human monoclonal antibodies with the desired specificity may be difficult and the level of production from human cell lines is often low, precluding their development.

An alternative approach involves the production of human-mouse chimeric antibodies in which the genetic information encoding the murine heavy and light chain variable regions are fixed to genes encoding the human heavy and light constant regions. The resulting mouse-human hybrid has about 30% of the intact immunoglobulin derived from murine sequences. Therefore, although a number of laboratories have constructed chimeric antibodies with mouse

variable and human constant domains (10-18), the mouse variable region may still be seen as foreign (19).

SUMMARY OF THE INVENTION

It is therefore an object of the present invention to provide a complementarity determining region (CDR)-grafted human antibody which contains at least one CDR from each variable heavy chain and variable light chain of at least one murine monoclonal antibody, against the RSV antigen. Preferably, the murine monoclonal antibody is a neutralizing antibody. It is also preferable that said murine antibody is an antibody against RSV F antigen.

The term "animal" as used herein is used in its broadest sense includes mammals including humans.

DETAILED DESCRIPTION OF THE DRAWINGS

The drawings depicted and described herein are intended to further illustrate the present invention and are not intended to limit the invention in any manner whatsoever.

Figure 1 shows the amino acid (AA) sequence design of CDR-Grafted anti-RSV F glycoprotein V_H. The figure depicts the AA sequence for the human HV3 V_H before grafting, CDR grafted V_H, and murine MAb1308F V_H from which the CDR sequence was grafted. The heavily underlined regions identify the CDR sequence which was grafted into the human HV3 V_H and each of the three regions is identified as CDR1, CDR2 and CDR3, respectively.

Figure 2 shows the amino acid (AA) sequence design of CDR-Grafted anti-RSV F Protein V_L. The figure depicts the AA sequence for the human K102 V_L before grafting, CDR grafted V_L, and murine MAb1308F V_L from which the CDR sequence was grafted. The heavily underlined regions identify the CDR sequence which was grafted into the human

K102 V_L and each of the three regions is identified as CDR1, CDR2 and CDR3, respectively.

Figure 3 depicts the oligonucleotides used to make Hu1308V_H, the sequences which are underlined are the specific primer sequences.

Figure 4 depicts the oligonucleotides used to make Hu1308V_L, the sequences which are underlined are the specific primer sequences.

Figure 5 depicts the plasmid construction of the expression vectors for Humanized 1308.

Figure 6 depicts a graph of the Neutraliziation of RSV as percent neutralization versus ng MAb per reaction for neutralizing with Cos Hu1308F and with Mu1308F.

DETAILED DESCRIPTION OF THE INVENTION

Applicants have found that transplantation into a human antibody, of only the genetic information for at least one CDR from each of the variable heavy and variable light chain derived from murine monoclonal antibody against RSV antigen, is effective for the prevention and treatment of RSV in animals. Preferably the murine antibody is a neutralizing antibody against RSV. Another aspect of the present invention provides for the murine antibody to be an antibody against RSV F antigen. Preferably, the murine antibody is neutralizing antibody against RSV F antigen. The substitution of the mouse CDR's into the human variable framework segments minimizes the potential for human anti-mouse antibody (HAMA) responses while retaining binding affinity and specificity for antigen, RSV F protein. Since, the CDR's do not contain characteristic murine or human motifs, the human antibodies containing the murine antibody CDR's are essentially indistinguishable from completely human antibodies, thereby, minimizing the human antibody

response while retaining binding affinity and specificity for RSV F antigen.

The development of a humanized antibody against RSV F antigen began with a murine antibody against RSV F antigen. Examples of murine antibodies of this type are: MAb 1436C, MAb 113, MAb 112, MAb 151, MAb 1200, MAb 1214, MAb 1237, MAb 1129, MAb 1121, MAb 1107, MAb 131-1, MAb 43-1, MAb 1112, MAb 1269, MAb 1243, MAb 1331H, MAb 1308F and MAb 1302A (see citation 21).

An aspect of the present invention provides that the CDRs of the human antibody are comprised of three complementarity determining regions (CDRs) from each variable heavy and variable light chain of the murine antibody.

The murine antibodies against RSV F antigen have been mapped by competitive binding and reactivity profiles of virus escape mutants to three broad antigenic sites (A, B, C) containing 16 distinct epitopes (20). The epitopes within antigenic sites A and C have shown the least variability in natural isolates.

Therefore, another aspect of this invention provides for a human antibody containing at least one CDR from each variable heavy and variable light chain of at least one murine antibody against RSV F antigen which is specific for antigenic site A or C. Preferably, this invention provides for the murine antibody against RSV F antigen specific for antigenic site C, where the murine antibody is MAb 1308F.

A preferred embodiment of this invention is a human antibody which contains CDR's of the variable heavy chain of murine antibody MAb 1308F against the RSV F antigen. The CDR variable heavy chain of MAb 1308F comprises three CDRs having the following amino acid sequences: Nos. 31 to 35, 47 to 60 and 99 to 106. In addition, this embodiment contains CDR's of a variable light chain of MAb 1308F of murine

antibody against RSV F antigen. The CDR variable light chain comprises three CDR's having the following amino acid sequences: Nos. 24 to 34, 50 to 56 and 89 to 97.

An additional aspect of applicants' invention is a process for preventing or treating RSV infection comprising administering to the animal an effective amount of a human antibody containing at least one CDR from each variable heavy and variable light chain, of at least one murine antibody against RSV F antigen.

Another aspect of applicants' invention is a composition comprising administering an effective amount of the human antibody as described above in conjunction with an acceptable pharmaceutical carrier. Acceptable pharmaceutical carriers include but are not limited to non-toxic buffers, fillers, isotonic solutions, etc.

The composition of Applicant's invention may be administered topically or systemically. Examples of topical administration are intranasal administration and inhalation of an aerosol containing the human antibody composition. Systemic administration may be accomplished by intravenous or intramuscular injection of the human antibody composition.

A preferred aspect of Applicants' invention is that the human antibody is administered as part of a plurality of human antibodies against RSV F antigen. These antibodies can be against the same or different epitopes of the RSV F antigen.

Additionally, the human antibody of this invention can be used clinically for diagnosing respiratory syncytial virus in patients. Because of their affinity for RSV F antigen these human antibodies can be used in known diagnostic assay procedures for detecting the presence and concentration of RSV F antigen cells in samples, e.g., body

fluids. The human antibodies of the present invention can for example be attached or bound to a solid support, such as latex beads, a column, etc., which are then contacted with a sample believed to contain RSV F antigen.

Applicants' development of human antibodies against RSV, began with murine hybridoma cells producing murine monoclonal antibodies which have been shown to neutralize RSV in vitro and protect cotton rats against lower respiratory tract infection with RSV.

One such antibody was selected, which is specific for antigenic site C, to produce mouse-human chimeric antibodies. This antibody was chosen on the basis that it: (i) reacted with a large number of virus strains tested (at least 13 out of 14 isolated); (ii) retained neutralizing activity against virus escape mutants selected with other anti-F antibodies and (iii) blocked RSV replication when administered at low doses to cotton rats by intranasal route prior to virus challenge. The antibody showed significant reduction in pulmonary virus titer among antibodies in that respective region. Murine antibody 1308F, specific for the C region of RSV F protein, was chosen as the initial target for humanization.

In summary, the human antibodies were constructed as follows: the RNA was extracted from the murine antibody-producing cell line, the murine variable regions which are responsible for the binding of the antibody to RSV were cloned and sequenced, resulting in the identification of the murine antibody CDRs. Then a human variable heavy and light chain framework sequence having the highest homology with the variable heavy and light chain murine antibody, was selected. A human framework sequence such as described above is best able to accept the murine-derived CDRs.

The murine 1308F variable heavy chain was compared to various human germline genes, the highest homology was to the human germline gene HV3. The two sequences were 62% homologous overall and 65% in the framework regions. Significantly, there is good homology at the junctions of the CDR segments and the frameworks with the exception of the 5' end of FR2. The murine derived variable heavy chain CDRs were then substituted into the variable heavy chain human germline gene HV3. The mouse and human sequences as well as that of a potential CDR-Grafted combination of the two is shown in Figure 1.

A similar analysis of the V_L region revealed high homology to the human germ line V-Kappa gene K 102. The alignment of these sequences is shown in Figure 2. In this case the homology is 62% overall and 73% in the framework regions. The murine-derived variable light CDRs were then substituted into the human variable light chain of human germline gene K102.In each case a human J-region can be selected which is identical to the mouse sequence.

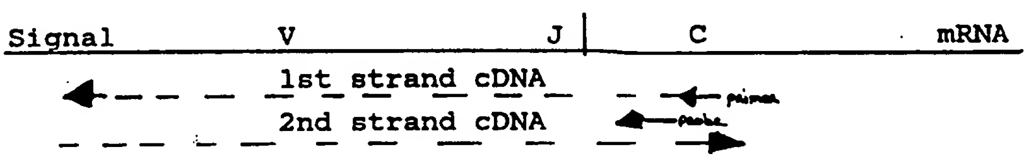
Therefore, human antibodies are expressed and characterized relative to the parental murine antibodies to be certain that the genetic manipulation has not drastically altered the binding properties of the antibodies.

Applicants present herein examples which are further illustrative of the claimed invention but not intended to limit the invention.

Examples 1

cDNA cloning and sequencing of anti-RSV F Protein antibody 1308F

cDNA copies of the V_H and V_L of the target antibody were generated as follows. The first strand CDNA reaction was carried out using AMV reverse trenscriptase and a phosphorylated oligonucleotide primer complementary to a segment of the mRNA coding for the constant region of the particular heavy or light chain isotype. For 1308F the isotype is gammal, kappa and the specific oligonucleotides were 5'AGCGGATCCAGGGGCCAGTGGATAGAC complementary to codons 129-137 of the CH1 region of the murine Gammal gene, and 5'TGGATGGTGGGAAGATG complementary to codons 116-122 of the murine C-kappa gene. The primer anneals to a segment of the mRNA adjacent to the variable region. Second strand cDNA synthesis was carried out using RNase H and E. coli DNA polymerase I, as described by Gubler and Hoffman (Gene 25,;263, 1983), followed by T4 DNA polymerase to assure that blunt ends are produced.



The ds-cDNA was ligated into pUC18 which had been digested with restriction endonuclease SmaI and treated with alkaline phosphatase. The ligation was used to transform E. coli DH5a by the method of Hanahan (J. Mol. Biol. 166;557, 1983). Oligonucleotide probes corresponding to C-region sequence lying between the first strand cDNA primer and the V-region were used in colony hybridizations to identify

transformants carrying the desired cDNA segment. The specific probe sequences were GGCCAGTGGATAGAC complementary to codons 121-125 of murine CH1 regions and TACAGTTGGTGCAGCA complementary to codons 110-115 of c-Kappa, respectively. Candidate plasmids, isolated from colonies which were positive in the hybridization, were analyzed by digestion with restriction endonucleases Eco RI and Hind III to release the cDNA insert. Those with inserts of 400-500bp were subjected to DNA sequencing.

The cDNA inserts were inserted into M13 mp18 and mp19 for the determination of the DNA sequence on both strands. Single stranded DNA from the resulting recombinant bacteriophage was isolated and sequenced by the dideoxy chain termination method (Proc. Nat. Acad. Sci. USA 74; 5463, 1977).

In order to confirm that the pair of rearranged and somatically mutated V gene cDNA's isolated from the 1308F hybridoma represented those which were in the 1308F antibody, a single-chain Fv gene was generated, expressed in and secreted from mammalian cells, then assayed for binding to RS virus. Competition binding experiments then were used to demonstrate the identity of the binding site.

Example 2

Design and assembly of human 1308F V_H and V_L

The CDR regions of the V_H and V_L were identified by comparing the amino acid sequence to known sequences as described by Kabat (38). In order to select the human framework sequences best able to accept the mouse derived CDR sequences in a conformation which retains the structure of the antigen combining site, the following strategy was employed. First, the sequence of the murine V_H and V_L regions will be compared to known human sequences from both

the Genbank and NBRF protein databanks using the Wordsearch program in the Wisconsin package of sequence manipulation programs (Nucleic Acid Res. 12;387). The best several human V-regions were then analyzed further on the basis of similarity in the framework regions, especially at the junctions of the framework and CDR regions (see Figures 1 and 2).

The CDR-grafted V_H region together with the respective leader sequence of the human v-region gene was synthesized de novo using four overlapping oligonucleotides ranging from 100-137 nucleotides in length (see Figure 3). The oligonucleotides were first allowed to anneal in pairwise combinations and extended with DNA polymerase to generate approximately 200bp ds DNA fragments with an overlapping region. the fragments were then mixed and subjected to PCR using primers at the 3'end of one fragment and the 5' end of the other fragment. The only product which can be formed under these condition is the full length V_H segment. The specific primer sequences are underlined in Figure 3. An endonuclease Sac I site was included at the 3' end of the V_H sequence in order to join it to a human constant region gene segment.

The CDR-grafted V_L region was synthesized in a similar way (see Figure 4). In this instance the initial 200bp fragments were amplified separately and inserted into separate plasmeds. The fragment coding for the amino terminus was cloned into a pUC18 derivative as an NcoI-SmaI fragment while the fragment coding for the carboxyl-terminus was cloned as a SmaI to Hind III fragment. The fragments were subsequently combined via a SmaI site at the junction. The oligonucleotides are indicated in Figure 4. A Hind III site was included near the 3' end of the gene segment in order to join it to a human C-kappa gene.

Example 3

Construction of Vectors for 1308F expression

The NcoI-SacI fragment representing the humanized V_H was joined to a SacI -Notl fragment representing a human c-Gamma I CDNA and inserted into pS 18 (which is pUC 1 8 with Ncol and NotI restriction sites incorporated into the polylinker region between the BamHI and Kpnl sites). The humanized 1308F-gammal gene on a SacI-NotI fragment was then combined with a Pvul-NotI fragment from pSJ37 carrying a poly A addition site and a PvuI-SacI fragment from pSV2-dhfr-pCMV containing the SV40 origin of replication, a dhfr gene and the CMV immediate early promoter. The resulting plasmid was designated pSJ60.

The NcoI-HindIII fragment representing the humanized V_L was joined to a HindIII-Notl fragment representing a human c-Kappa CDNA in pS18. The humanized 1308F-Kappa gene on a SalI-NotI fragment was then combined with a Pvul-NotI fragment from pSJ37 carrying a poly A addition site and a PvuI-SalI fragment from pSV2-dhfr-pCMV, containing the SV40 origin of replication, a dhfr gene and the CMV immediate early promoter. The resulting plasmid was designated pSJ61.

Finally pSJ60 and pSJ61 were combined into a single plasmid containing both the light and heavy chains and expression signals. This was accomplished by isolating a PvuI-Bam HI fragment from pSJ61 carrying the light chain with a Pvu I - Bgl II fragment from pSJ60 carrying the heavy chain to generate pSJ66. (See Figure 5).

Example 4

Transfection of Cosl cells with PSJ60 and PSJ61

Transfections were carried out according to the method of McCutchan and Pagano (J. Nat. Can. Inst. 41: 351-356, 1968) with the following modifications. COS 1 cells (ATCC

CRL1650) were maintained in a humidified 5% CO2 incubator in 75 cm^2 tissue culture flasks in Dulbecco's Modified Eagle Medium (DMEM, GIBCO #320-1965) supplemented with 10% Fetal Bovine Serum (FBS, GIBCO #200-6140) and 2mM L-glutamine (BRL #320-5030) and passed at a split ratio of 1:20 when the cells had reached confluence. 48 hours prior to transfection, 5 100mm tissue culture dishes were seeded with 1.5×10^6 cells per dish in 12ml DMEM, 10% FBS, 2mM L-glutamine, 1% penicillin-streptomycin (P-S, GIBCO #600-5070). The day of the transfection, 120 ug each of the plasmids pSJ60 and pSJ61 were combined, ethanol precipitated, and aseptically resuspended in 2.5ml Tris-Buffered-Saline. The resuspended DNA was added dropwise, with mixing, to 10ml of DMLEM containing 1 mg/ml DEAE-dextran (Phamiacia #17-0350-01) and 250 uM chloroquine (Sigma #C6628). The medium was removed from the COS1 cells in the 100 mm dishes and the cells were washed once with Dulbecco's phosphate buffered saline (D-PBS, GIBCO #310-4190), and 2.5ml DMEM supplemented with 10% NuSerum (Collaborative Research #55000) were added to each plate. 2.5ml of the DNA/DEAE-dextran/chloroquine mix were added dropwise to each plate, the plates swirled to mix the DNA, and were returned to the incubator. After 4 hours in the incubator, the supernatant was aspirated from the cells and the cells were washed once with 5ml D-PBS. The cells were shocked for 3 minutes by the addition of 5ml of 10% dimethylsulfoxide (DMSO) in D-PBS at room temperature. The DMSO was aspirated from the cells and the cells were washed with 5ml D-PBS. 14ml of DMEM/10% FBS/2mM L-glutamine/1%P-S were added to each plate and the plates were returned to the incubator.

Three days post-transfection the medium was removed from the plates, pooled, and stored at -20°C. The cells

were harvested, pooled, and seeded into 4 150cm^2 tissue culture flasks two with 40ml DMEM/10% NuSerum and two with 40ml DMEM/10% FBS/2mM L-glutamine. The medium was collected and the cells refed at 7, 10, and 14 days. In this way a total of 125ug of humanized 1308F antibody was accumulated in 310ml of medium supplemented with FBS and 85ug in 240ml of medium supplemented with NuSerum.

Example 5

Transfections of COS 1 cells with PSJ66

48 hours prior to transfection, 5 100mm tissue culture dishes were seeded with 1.5 x 10^6 cells per dish in 12ml DMEM, 10% FBS, 2mM L-glutamine, 1% penicillin-streptomycin (P-S, GIBCO #600-5070). The day of the transfection, 125ug of the plasmid pSJ66 were ethanol precipitated and aseptically resuspended in 1.0 ml Tris-Buffered-Saline. The resuspended DNA was added dropwise, with mixing, to 4.0ml of DMEM containing 1mg/ml DEAE-dextran (Pharmacia #17-0350-01) and 250uM chloroquine (Sigma #C6628). The medium was removed from the COS1 cells in the 100mm dishes and the cells were washed once with Dulbecco's phosphate buffered saline (D-PBS, GIBCO #310-4190), and 2.5ml DMEM supplemented with 10% NuSerum (Collaborative Research #55000) were added to each plate. 2.5ml of the DNA/DEAE-dextran/chloroquine mix were added dropwise to each plate, the plates swirled to mix the DNA, and were returned to the incubator. After 4 hours in the incubator, the supernatant was aspirated from the cells and the cells were washed once with 5ml D-PBS. The cells were shocked for 3 minutes by the addition of 5ml of 10% dimethylsulfoxide (DMSO) in D-PBS at room temperature. The DMSO was aspirated from the cells and the cells were washed with 5ml D-PBS. 14ml of DMEM/10% FBS/2mM

L-glutamine/1%P-S were added to each plate and the plates were returned to the incubator.

Three days post-transfection the medium was removed from the plates, pooled, and stored at -20°C. The cells were harvested, pooled, and seeded into 4 150cm^2 tissue culture flasks two with 40 ml DMEM10% NuSerum and two with 40 ml DMEM10% FBS/2mM L-glutamine. The medium was collected and the cells refed at 7, 10, and 14 days. In this way a total of 190ug of humanized 1308F antibody was accumulated in 310ml of medium supplemented with FBS and 120ug in 240ml of medium supplemented with NuSerum.

The concentration of humanized 1308F antibody secreted from the Cos1 cells into the medium was determined using a capture ELISA. Goat anti-human IgG Fc coated onto 96 well plates was used to capture the humanized antibody. Peroxidase conjugated goat anti-human whole IgG developed with a chromogenic substrate was then used to detect the bound antibody. A purified human IgG1/Kappa preparation was used to calibrate the assay.

Example 6

Neutralization of RSV with humanized 1308F

METHODS:

RSV was neutralized with either humanized 1308F from Cos cell supernatant or purified 1308F murine monoclonal antibody. This was done by incubating 50 plaque-forming units of RSV with serial 2-fold dilutions of antibody for 1.0 hour at 37°C. Confluent monolayers of Hep2 cells in 24 well panels were infected with 100µl of antibody treated virus, untreated control virus, and mock infected controls. Incubated for 1.5 hours at 37°C, humidified , and 5% CO_2 and overlayed with 1.5mL EMEM, 1% FBS, and 1% methyl cellulose. Cells were fixed and stained with glutaldehyde and crystal

violet on day 4. Plaques were counted in triplicate wells and plotted as percent neutralization. The results shown in Figure 6 indicate that both the purified murine 1308F monoclonal and the humanized 1308F monoclonal antibody at 5 to 10 ng per well yield similar 50% reductions in RSV plaques.

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WHAT IS CLAIMED IS:

1. A human-murine chimeric antibody, comprising:

a human antibody containing at least one CDR from each of the variable heavy and variable light chains of a murine monoclonal antibody against RSV.

2. An antibody as in Claim 1, wherein said murine monoclonal antibody is a neutralizing antibody against RSV.

3. An antibody as in Claim 1, wherein said murine monoclonal antibody is an antibody against RSV F protein.

4. An antibody as in Claim 3, wherein said murine monoclonal antibody is a neutralizing antibody against RSV F protein.

5. An antibody as in Claim 3, wherein:

said CDR comprises three complementarity determining regions from each of said variable heavy and variable light chains.

6. An antibody of Claim 5 wherein said murine antibody against RSV F protein is specific for antigenic site A of said protein.

7. A human antibody of Claim 5 wherein said murine antibody against RSV F protein is specific for antigenic site C of said protein.

8. A human antibody of Claim 7 wherein said murine antibody is MAb 1308F.

9. A human antibody as in Claim 8, wherein:

said three complementarity determining regions from said variable heavy chain of Mab 1308F comprise amino acid sequence Nos. 31 to 35, 47 to 60 and 99 to 106 and said three complementarity determining regions from said variable light chain of MAb 1308F comprise amino acid sequence Nos. 24 to 34, 50 to 56 and 89 to 97.

10. A process for preventing or treating a respiratory syncytial virus infection in an animal comprising:

administering to said animal an effective amount of a human antibody which contains at least one CDR from each variable heavy chain and variable light chain, of at least one murine monoclonal antibody against respiratory syncytial virus F protein.

11. The process of Claim 10 wherein:

said CDR's have three complementarity determining regions from each of said variable heavy and variable light chains.

12. A composition for preventing or treating respiratory syncytial virus infection in an animal comprising:

(a) an effective amount of a human antibody which contains at least one CDR from each variable heavy and variable light chains of at least one murine monoclonal antibody against respiratory syncytial virus F protein, and

(b) an acceptable pharmaceutical carrier.

13. A process for preventing or treating a respiratory syncytial virus infection in an animal comprising:

administering to said animal an effective amount of a plurality of human antibodies which contain at least one CDR from each variable heavy and variable light chain of at least one murine monoclonal antibody against RSV F protein.

FIG. 1A

DESIGN OF CDR-GRAFTED ANTI-RSV F PROTEIN V_H

```
                    5                   10
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys
 *              *                        *   *
Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val

                    25                  30
Ser Cys Lys Ala Ser Gly Tyr Thr Phe Asn Ser Tyr
                         *   *   *   *   *
Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys Asp Tyr
Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys Asp Pyr

                    45                  50
Pro Gly Gln Gly Leu Glu Trp Met Gly Ile Ile Asn
                             *           *   *
Pro Gly Gln Gly Leu Glu Trp Ile Gly Trp Ile Asp
     *                                    CDR
Pro Glu Gln Gly Leu Glu Trp Ile Gly Trp Ile Asp

                    65                  70
Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg
 *   *
Asp Pro Lys Phe Gln Gly Arg Val Thr Met Thr Arg
                         *   *   *   *       *
Asp Pro Lys Phe Gln Gly Lys Ala Ser Ile Thr Ser

                    85                  90
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala
 *                       *
Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala

                    105                 110
 -   -   -   -   -   -   -   -   -   -   -   -

Thr Ser Ser Phe Asp Phe Trp Gly Gln Gly Thr Thr
    CDR3
Thr Ser Ser Phe Trp Gly Gln Gly Thr Thr Leu Thr
```

MATCH WITH FIG. 1B

/ J>>

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FIG. 1B

MATCH WITH FIG. 1A

```
               15                                     20
Lys  Pro  Gly  Ala  Ser  Val  Lys  Val  Human HV3  VH

L s  Pro  Gly  Ala  Ser  Val  Lys  Val  "CDR Grafted"
 *                   *    *                  VH
Arg  Pro  Gly  Ala  Leu  Val  Lys  Leu  Murine 1308F
                                              VH

               35                          40
Tyr  Met  His  Trp  Val  Arg  Gln  Ala
      *    *
Tyr  Ile  Tyr  Trp  Val  Arg  Gln  Ala
     CDR  1                 *          *
Tyr  Ile  Tyr  Trp  Val  Lys  Gln  Arg

               55                          60
Pro  Ser  Gly  Gly  Ser  Thr  Ser  Tyr
      *    *         *         *    *
Pro  Glu  Asn  Gly  Asn  Thr  Val  Phe
2
Pro  Glu  Asn  Gly  Asn  Thr  Val  Phe

               75                          80
Asp  Thr  Ser  Thr  Ser  Thr  Val  Tyr

Asp  Thr  Ser  Thr  Ser  Thr  Val  Tyr
      *    *                   *
Asp  Thr  Ser  Ser  Asn  Thr  Ala  Tyr

               95                         100
Val  Tyr  Tyr  Cys  Ala

Val  Tyr  Tyr  Cys  Ala  Tyr  Tyr  Gly

Val  Tyr  Tyr  Cys  Ala  Tyr  Tyr  Gly
                   <<v    /        D

               115
 _    _    _    _    _

Leu  Thr  Val  Ser  Ser

Val  Ser  Ser
```

DESIGN OF CDR-GRAFTED ANTI-RSV F PROTEIN V_L

```
                    5                               10
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser

Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser
         *                           *   *   *
Asp Ile Lys Met Thr Gln Ser Pro Ser Ser Met Tyr

                    25                              30
Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Trp
             *               *       *   *   *
Ile Thr Cys Lys Ala Ser Gln Asp Ile Asn Arg Tyr
            -----------------------------------
                                 CDR 1
Ile Thr Cys Lys Ala Ser Gln Asp Ile Asn Arg Tyr

                    45                              50
Gly Lys Ala Pro Lys Leu Leu Ile Tyr Asp Ala Ser
                                     *       *
Gly Lys Ala Pro Lys Leu Leu Ile Tyr Arg Ala Asn
         *           *           *  -----------
Gly Lys Ser Pro Lys Thr Leu Ile His Arg Ala Asn

                    65                              70
Arg Phe Ser Gly Ser Gly Ser Gly Thr Glu Phe Thr

Arg Phe Ser Gly Ser Gly Ser Gly Thr Glu Phe Thr
                                 *       *   *
Arg Phe Ser Gly Ser Gly Ser Gly Gln Glu Tyr Ser

                    85                              90
Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn
                                 *       *   *
Asp Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Phe His
 *       *   *   *              ---------------
Glu Asp Met Gly Ile Tyr Tyr Cys Leu Gln Phe His

                    105
 -   -   -   -   -   -   -

Gly Thr Lys Leu Glu Ile Lys

Gly Thr Lys Leu Glu Ile Lys
```

MATCH WITH FIG. 2B

FIG. 2A

FIG. 2B

```
        15                   20
Ala Ser Val Gly Asp Arg Val Thr - Human K102
                                    VL
Ala Ser Val Gly Asp Arg Val Thr - "CDR Grafted"
 *       *       *                  VL
Val Ser Leu Gly Glu Arg Val Thr - Murine 1308F
                                    VL

        35                  40
Leu Ala Trp Tyr Gln Gln Lys Pro
     *
Leu Asn Trp Tyr Gln Gln Lys Pro
             *
Leu Asn Trp Phe Gln Gln Lys Pro

        55                  60
Ser Leu Glu Ser Gly Val Pro Ser
 *       *   *
Arg Leu Val Asp Gly Val Pro Ser
CDR 2
Arg Leu Val Asp Gly Val Pro Ser

        75                  80
Leu Thr Ile Ser Ser Leu Gln Pro

Leu Thr Ile Ser Ser Leu Gln Pro
                             *
Leu Thr Ile Ser Ser Leu Glu Phe

        95                 100
Ser Tyr Ser  -   -   -
 *   *   *
Glu Phe Pro Tyr Thr Phe Gly Gly
  CDR 3
Glu Phe Pro Tyr Thr Phe Gly Gly
     <<v  /  J>>
```

MATCH WITH FIG. 2A

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FIG.3A

Oligos used to make Hu1308 V_H

```
5'                                  3'
 gcgaattccatggactggacctggagggtc
         MetAspTrpThrPrpArgValPheCysLeuLeuAlaValAlaProGlyAlaHisSerGln
5'  ccATGGACTGGACCTGGAGGGTCTTCTGCTTGCTGGCTGTAGCACCAGGTGCCCACTCCCAG
        1---------+---------+---------+---------+---------+
3'      TACCTGACCTGGACCTCCCAGAAGACGAACGACCGACATCGTGGTCCACGGGTGAGGGTC

        ValGlnLeuValGlnSerGlyAlaGluValLysLysProGlyAlaSerValLysValSer
        GTGCAGCTGGTGCAGTCTGGAGCTGAGGTGAAGAAGCCTGGAGCCTCAGTGAAGGTTTCC
     61---------+---------+---------+---------+---------+---------+
        CACGTCGACCACGTCAGACCTCGACTCCACTTCTTCGGACCTCGGAGTCACTTCCAAAGG

        CysLysAlaSerGlyPheAsnIleLysAspTyrTyrIleTyrTrpValArgGlnAlaPro
        TGCAAGGCATCTGGATTCAACATTAAGGACTACTACATTTACTGGGTGCGACAGGCTCCT
    121---------+---------+---------+---------+---------+---------+
        ACGTTCCGTAGACCTAAGTTGTAATTCCTGATGATGTAAATGACCCACGCTGTCCGAGGA

        GlyGlnGlyLeuGluTrpMetGlyTrpIleAspProGluAsnGlyAsnThrValPheAsp
        GGACAAGGGCTCGAGTGGATGGGTTGGATTGACCCTGAGAATGGTAATACTGTGTTTGAC
    181---------+---------+---------+---------+---------+---------+
        CCTGTTCCCGAGCTCACCTACCCAACCTAACTGGGACTCTTACCATTATGACACAAACTG
```

MATCH WITH FIG. 3B

MATCH WITH FIG. 3A

```
    ProLysPheGlnGlyArgValThrMetThrArgAspThrSerThrSerThrValTyrMet
    CCGAAGTTCCAGDGCAGAGTCACCATGACCAGGGACACGTCCACGAGCACAGTCTACATG
241 ---------+---------+---------+---------+---------+---------+
    GGCTTCAAGGTCCCGTCTCAGTGGTACTGGTCCCTGTGCAGGTGCTCGTGTCAGATGTAC

    GluLeuSerSerLeuArgSerGluAspThrAlaValTyrTyrCysAlaTyrTyrGlyThr
    GAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCGTACTACGGTACA
301 ---------+---------+---------+---------+---------+---------+
    CTCGACTCGTCGGACTCTAGACTCCTGTGCCGGCACATAATGACACGCATGATGCCATGT

    SerSerPheAspPheTrpGlyGlnGlyThrThrLeuThrValSerSer
    AGCTCCTTTGACTTCTGGGGCCAAGGCACCACTCTCACAGTGAGCTCA
361 ---------+---------+---------+---------+--------
    TCGAGGAAACTGAAGACCCCGGTTCCGTGGTGAGAGTGTCACTCGAGTattcctagg 5'
                                 ggtgagagtgtcactcgagtattcctagggc
                                3'                                5'
```

FIG. 3B

Oligos usedto make Hu 1308 V_H

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FIG. 4A

Oligos usedto make Hu 1308 V_L

```
cgcggatccatggacatgagggtcccc
      MetAspMetArgValProAlaGlnLeuLeuGlyLeuLeuLeuLeuTrpLeuProGlyAla
    ccATGGACATGAGGGTCCCCGCTCAGCTCCTGGGGCTCCTGCTGCTCTGGCTCCCAGGTGCC
  1   ---------+---------+---------+---------+---------+---------+
      TACCTGTACTCCCAGGGGCGAGTCGAGGACCCCGAGGACGACGAGACCGAGGGTCCACGG

      LysCysAspIleGlnMetThrGlnSerProSerThrLeuSerAlaSerValGlyAspArg
      AAATGTGATATCCAGATGACCCAGTCTCCTTCCACCCTGTCTGCATCTGTAGGAGACAGA
 61   ---------+---------+---------+---------+---------+---------+
      TTTACACTATAGGTCTACTGGGTCAGAGGAAGGTGGGACAGACGTAGACATCCTCTGTCT

      ValThrIleThrCysLysAlaSerGlnAspIleAsnArgTyrLeuAsnTrpTyrGlnGln
      GTCACCATCACTTGCAAGGCGAGTCAGGACATTAATAGGTATTTAAACTGGTACCAGCAG
121   ---------+---------+---------+---------+---------+---------+
      CAGTGGTAGTGAACGTTCCGCTCAGTCCTGTAATTATCCATAAATTTGACCATGGTCGTC

      LysProGlyLysAlaProLysLeuLeuIleTyrArgAlaAsnArgLeuValAspGlyVal
      AAACCCGGGAAAGCCCCTAAGCTCCTGATCTATCGTGCAAACAGATTGGTAGATGGGGTC
181   ---------+---------+---------+---------+---------+---------+
      TTTGGGCCCTTTCGGGGATTCGAGGACTAGATAGCACGTTTGTCTAACCATCTACCCCAG
```

MATCH WITH FIG. 4B

MATCH WITH FIG. 4A

```
     ProSerArgPheSerGlySerGlySerGlyThrGluPheThrLeuThrIleSerSerLeu
     CCATCAAGGTTCAGCGGCAGTGGATCTGGGACAGAATTCACTCTCACCATCAGCAGCCTG
241  ---------+---------+---------+---------+---------+---------+
     GGTAGTTCCAAGTCGCCGTCACCTAGACCCTGTCTTAAGTGAGAGTGGTAGTCGTCGGAC

     GlnProAspAspPheAlaThrTyrTyrCysLeuGlnPheHisGluPheProTyrThrPhe
     CAGCCTGATGATTTTGCAACTTATTACTGCCTACAGTTTCATGAGTTTCCGTACACGTTC
301  ---------+---------+---------+---------+---------+---------+
     GTCGGACTACTAAAACGTTGAATAATGACGGATGTCAAAGTACTCAAAGGCATGTGCAAG
                                                        3' gtgcaag

     GlyGlyGlyThrLysLeuGluIleLys
     GGAGGGGGACCAAGCTTGAAATAAAA 3'
361  ---------+---------+------
     CCTCCCCCTGGTTCGAACTTTATTTT 5'
     cctccccctggttcgaaccc 5'
```

FIG. 4B

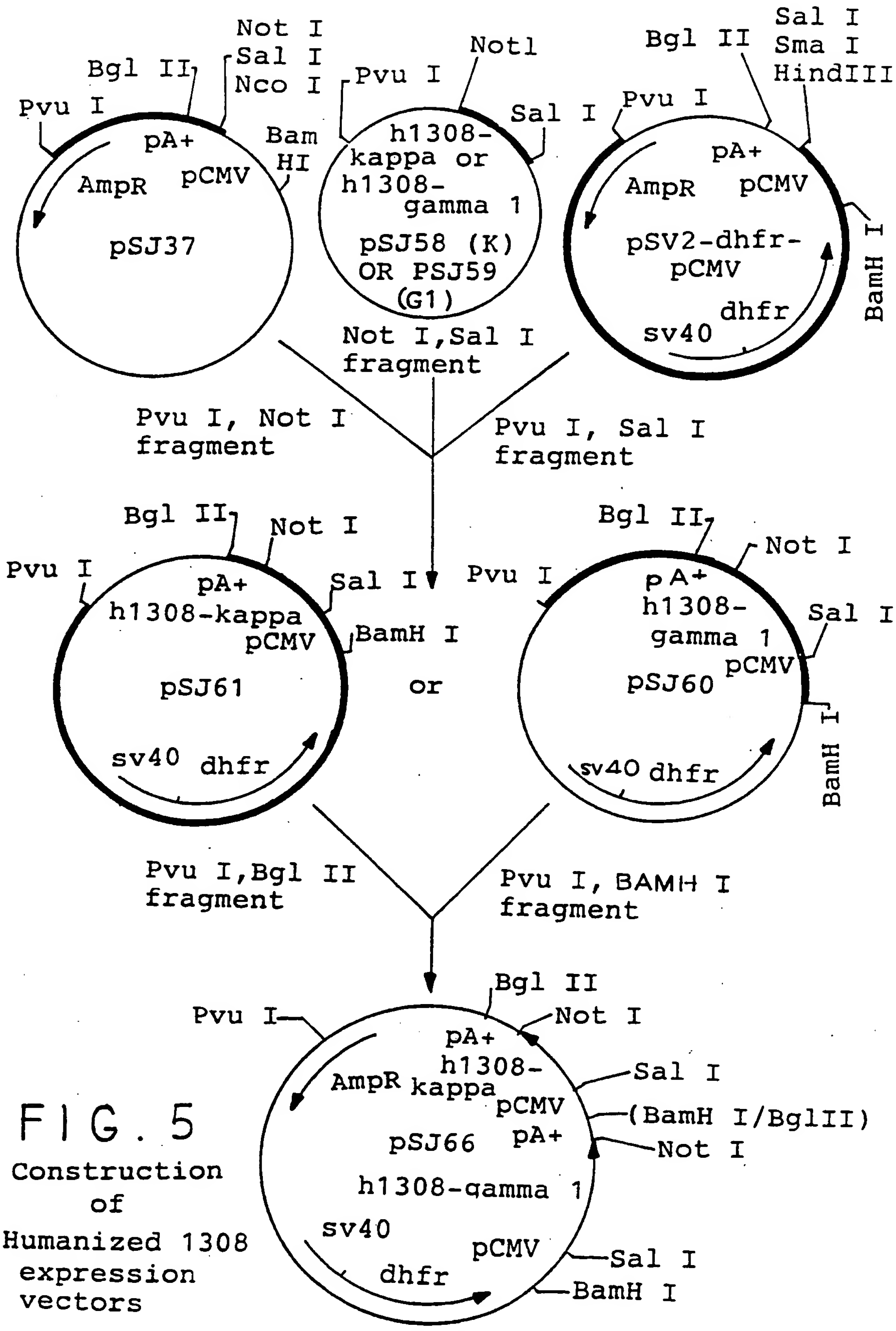
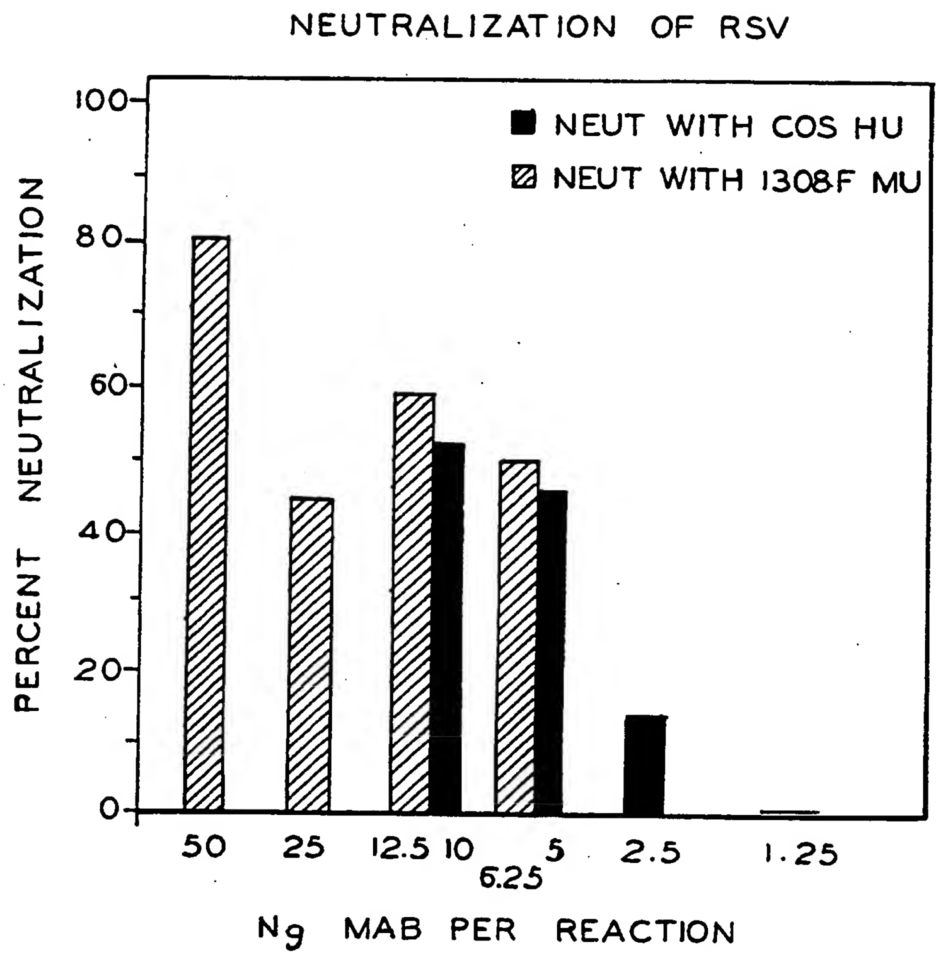


FIG. 5

Construction of Humanized 1308 expression vectors

FIG. 6



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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/01168

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C07K 15/28; C12N 5/20, 5/28; C12P 21/08; A61K 39/42, 39/395
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/389.4, 388.15, 387.3; 435/240.27, 70.21; 424/85.8, 86; 935/95, 96, 99

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	Biotechnology, Volume 9, Number 3, issued March, 1991, P.R. Tempest et al., "Reshaping a human monoclonal antibody to inhibit human respiratory syncytial virus infection in vivo", pages 266 to 271, see entire document.	1-5,12 6-11,13
Y	Developmental and Biological Standarization, Volume 57, issued 1984, E.J. Stott et al., "The characterization and uses of monoclonal antibodies to respiratory syncytial virus", pages 237 to 244, see entire document.	1-13
Y	Nature, Volume 321, issued 29 May 1986, P.T. Jones et al., "Replacing the complementarity-determining regions in a human antibody with those from a mouse", pages 522 to 525, see entire document.	1-13

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
01 April 1993	14 APR 1993

Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer Dyna Kryza for JACQUELINE G. KRIKORIAN
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/01168

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

530/389.4, 388.15, 387,3; 435/240.27, 70.21; 424/85.8, 86; 935/95, 96, 99

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

CAS Online, Biosis, Medline, WPI, APS, Genbank
search terms: antibodies, RSV-F protein, respiratory syncytial virus, treatment, chimeric human-mouse antibodies, sequences dislcosed